Corn Earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae) and Other Insect Associated Resistance in the Maize Inbred Tex6

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ABSTRACT A 2-yr field and laboratory study investigated insect resistance of the maize, *Zea mays* L., inbred Tex6, which has previously demonstrated resistance to *Aspergillus* ear rot and aflatoxin production, relative to susceptible inbred B73. Field studies indicated significantly greater resistance to insect feeding of V4–V8 growth stage Tex6 plants compared with B73 plants in both years, primarily to flea beetles (*Chaetonema* spp.). Field studies of natural (1999) and artificial (2000) infestations of corn earworms, *Helicoverpa zea* (Boddie), indicated much lower levels of kernel damage at milk stage (approximately three-fold) and smaller surviving larvae (approximately three-fold) in Tex6 compared with B73 ears. At harvest similar trends in reduction of numbers of damaged kernels per ear, as well as incidence and numbers of kernels per ear symptomatically infected by *Fusarium* spp. were noted. Laboratory studies indicated little difference in mortality or survivor weight of caterpillars or sap beetle adults caged with milk stage kernels of the two inbreds. However, assays with silks indicated significantly greater mortality of *H. zea* in both 1999 and 2000, and European corn borer, *Ostrinia nubilalis* (Hübner) in 1999 (only year tested) when fed Tex6 silks compared with B73 silks. Pollinated Tex6 silks were generally darker colored and more toxic than unpollinated silks. Thus, it is possible that commercially usable inbreds with resistance to insects, which also contribute to the mycotoxin problem through vectoring and damage, could be produced using Tex6 as a source.

KEY WORDS *Aspergillus*, *Helicoverpa zea*, plant resistance, corn, aflatoxin

THE PRESENCE OF ear mold toxins (mycotoxins) in U.S. corn causes hundreds of millions of dollars of direct and indirect losses each year (USDA-ARS 1999, Vardon 1998). One of the most insidious groups of mycotoxins are the aflatoxins, which are among the most potent carcinogens known (Lillehoj 1992). Considerable effort is being expended to control the producing fungi (primarily *Aspergillus flavus* Link) and the insects that contribute to the aflatoxin problem by vectoring the fungus or damaging corn which assists establishment of the fungus (Dowd 1998).

Corn ear resistance to *A. flavus* and/or production of aflatoxin is one management strategy that is being pursued to control aflatoxin. Several different inbreds or hybrids have been identified with varying levels of resistance to *A. flavus*, the aflatoxin-producing fungus found in corn, and/or aflatoxin production (e.g., Brown et al. 1998). The inbred Tex6, developed at the University of Illinois from a southern cultivar (Plant Introduction 401763), has relatively high and consistent resistance to both *Aspergillus* ear rot and aflatoxin production compared with the widely used inbred B73 (Hamblin and White 2000).

Common mechanisms may be involved in plant resistance to both insects and fungi, including secondary metabolites such as hydroxamic acids (e.g., Miller et al. 1996), directly active proteins such as ribosomal inactivating proteins (e.g., Dowd et al. 1998), or indirectly active proteins such as peroxidases (e.g., Dowd 1994b, Dowd and Lagrimini 1997). Common insect and fungal resistance quantitative trait loci (QTLs) have also been identified in maize (e.g., McMullen and Simcox 1995). Past studies involving an aflatoxin-resistant southern adapted maize inbred Mp313E (Scott and Zummo 1988) identified kernel resistance to insects as well (Dowd 1994a) which appeared to be at least partly due to the higher levels of peroxidases (Dowd 1994b). Peroxidases were subsequently confirmed as a resistance mechanism using studies involving transgenic plants (e.g., Privalle et al. 1999). We now report that in addition to resistance to *Aspergillus* ear rot/aflatoxin production, Tex6 also has resistance to insects relative to the inbred B73, especially silk resistance to the corn earworm, *Helicoverpa zea* (Boddie).

Materials and Methods

Plants. Seeds of Tex6 and B73 were obtained from seed increased from stock in 1998 (see Hamblin and

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White 2000). B73 is widely used as background material for commercial hybrids and is reported as having lower than average tolerance to European corn borer (e.g., Troyer 2001). Seedlings were initially germinated and grown under greenhouse conditions and transplanted at the three-leaf stage in late May to early June into a field site at Peoria, IL, using techniques described previously (Dowd 1994a, 2000). Supplemental high nitrogen fertilizer was added when leaves appeared pale, as described previously (Dowd 2000); three applications were made in 1999 and 2000 to V4, V6, and V8 (Ritchie and Benson 1989) plants. Both inbreds were planted at weekly intervals for 3 wk each year (80–100 seedlings per inbred per planting) starting at the same time, so that ears of each inbred would be available in milk stage during the same time period despite different rates of development. Different inbreds were planted in blocks to minimize cross pollination. Pollination was hand assisted, and examination of ears used in experiments indicated very little cross pollination, limited to only a few kernels on any ear (as indicated by yellow kernels on the white kerneled Tex6). No off color kernels were used in laboratory assays.

Insects. Corn earworms, fall armyworms [*Spodoptera frugiperda* (J.E. Smith)], and a corn feeding sap beetle (*Carpophilus freemani* Dobson) (Coleoptera: Nitidulidae) were reared on pinto bean diet as described previously (Dowd 1987, 1988; Dowd and Weber 1990). Adults of the dusky sap beetle, *Carpophilus lugubris* Murray (Coleoptera: Nitidulidae), were field collected using baited traps as described previously (Dowd 1994a, Dowd et al. 1998). European corn borer eggs, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), were provided by L.C. Lewis, USDA-ARS, Corn Insects and Genetics Research Laboratory, Ames IA, and hatched under the same conditions used to rear the other caterpillar species.

Field Experiments. Field studies were conducted in both 1999 and 2000 at the same site in Peoria, IL. Plants were observed weekly for insect damage. When obvious leaf damage was observed, the number of affected plants was recorded for each inbred. Staggered weekly planting affected infestation rates more in 1999 than 2000, hence a larger groups of plants of the same developmental stage (V4-V6) were rated in 2000 (minimum of 217) compared with 1999 (minimum of 72). Incidence of damaged plants was determined, and the presence of damaging insect species and characteristics of damage were observed. In 1999, the severity of damage was also determined by rating plants on a 0–10 scale based on the % of leaf consumed to the nearest 10% (1 = 10%, 2 = 20%, and so on).

In 1999, a high natural infestation of *H. zea* occurred, so additional insects were not added. At least 40 ears of each inbred were sampled at milk stage in 1999. In 2000, trapping in nearby areas indicated low populations of both *O. nubilalis* and *H. zea* moths during silking, so plants were artificially infested with *H. zea* to be consistent with the most commonly occurring insect species encountered in 1999. Thus, in 2000 the ears were infested as described previously (Dowd 2000), by adding 10 neonates to silks 7 d after pollination (all infestations were performed on the same day), and covering ears with cloth bags to retain caterpillars in the ear zone. At least 14 ears of each inbred were artificially infested and sampled at milk stage in 2000. Ears were evaluated for insect infestation and damage at milk stage and harvest stage, which involved natural insect infestations in both years (except milk stage samples in 2000). Evaluations at milk stage included measuring total ear length, length of silk channel from husk tip to ear tip, distance of caterpillar penetration in the silk channel from husk tip to ear tip, length of ear unfilled at the tip, numbers of kernels damaged, numbers and species of insects present (or by characteristic damage if absent per Dowd 2000), and weights of surviving caterpillars. At harvest, the incidence and numbers of kernels damaged by different insect species was determined. Because insect damage may facilitate invasion of fungi that produce mycotoxins (Dowd 1998), the incidence and numbers of kernels symptomatically infected by *Fusarium* spp. fungi (no *A. flavus* occurred in either year) was also determined. At least 20 ears of each inbred were sampled at harvest in both years of the study.

Laboratory Experiments. Silk and kernel evaluations for insect toxicity were performed concurrently with milk stage ear field evaluations. Milk stage kernels and silks from milk stage ears (consisting primarily of silks from pollinated kernels) were removed randomly from at least four different ears of each inbred and used for *ad libitum* studies with the different insect species. Dried silk ends were removed, and only the still turgid silk portions contained under the husk were used. Separating the silks by "parting" at the zone where filled and unfilled milk stage kernels conjoined indicated obvious color differences between silks (white to straw colored) attached to unpollinated kernels and silks (reddish brown) attached to pollinated kernels on the same ear of Tex6. No color differences between pollinated and unpollinated silks on milk stage ears were noted for B73 silks. Because of the different colored silks of Tex6 influenced by pollination state, which may reflect a different composition of toxins, assays were also set up using *H. zea* with corresponding silks of different pollination states from the same Tex6 ears as described for the other silk assays to determine if pollination state influenced relative resistance. All insect species were caged individually with single kernels or silk clippings using 24-well tissue culture plates, with 20 larvae per treatment (Dowd 1988, 1994b). Insects were examined daily and assays ended in 3–4 d (period of time material would remain fresh). Mortality was determined at each interval of examination (although only final values are reported). Survivors were killed by freezing and then weighed using a Mettler AE-163 analytical balance, which is accurate to 0.01 mg. Small larvae were transferred with a pin probe by gently touching the probe to the side of the larvae and then dislodged by gently touching the larvae to the weighing dish on the balance.

Table 1. Insect damage to young plant leaves in field studies of Tex6 and B73 maize inbreds at Peoria, IL, in 1999 and 2000

Inbred	% incidence	χ^2	*P*	*n*
1999				
Overall				
Tex6	31.9			72
B73	56.0	9.03	<0.01	84
Leaves with >1 damage rating				
Tex6	8.3			72
B73	45.8	26.1	<0.01	84
Leaves with >5 damage rating				
Tex6	4.2			72
B73	8.3	1.16	0.28	84
2000				
Tex6	8.8			217
B73	35.9	47.1	<0.01	234

Insect damage appeared primarily due to flea beetles (*Chaetonema* spp.). Degrees of freedom for chi-square analyses are all 1.

Statistical Analysis. Significant differences in mortality or other percentages were determined using the chi-square statistic of PROC FREQ (SAS Institute 1987). In cases where cell sizes were less than five, the log likelihood ratio statistic was used from this PROC. Significant differences in weights, kernels numbers, silk channel or ear fill distances, or distances of penetration were determined using analysis of variance (ANOVA) with PROC GLM or equivalent (SAS Institute 1987).

Results

Field Experiments. Younger (V4-V6 stage) Tex6 plants had a significantly lower incidence of insect damage (primarily flea beetle *Chaetonema* sp. as indicated by characteristic damage of narrow parallel lines [e.g., Gray 1999] and insect species present) (Table 1). The severity of damage was also significantly lower for Tex6 compared with B73 plants in 1999. The percentage of plants having a damage rating above 1.0 in 1999 was significantly higher for B73 (45.8%) compared with Tex6 plants (8.3%). There were no obvious differences in severity of damage in 2000, so this aspect was not rated.

Incidence of any caterpillar (all *H. zea*) presence on milk stage ears was similar for B73 and Tex6 inbreds in both years (Table 2). However, when *H. zea* were present, the number of *H. zea* per ear was significantly lower for Tex6 compared with B73 ears for both years. The number of kernels damaged per ear and weights of surviving caterpillars were both significantly lower for Tex6 compared with B73 ears in both years, and was not dependent on the distance from husk tip to filled kernels, as follows. *H. zea* penetrated Tex6 silk channels from husk tip to ear tip a significantly shorter distance compared with B73 silk channels in both years, although kernel damage occurred at about the same rate for Tex6 and B73 in 2000. The inbred with the longest silk channel from husk tip to ear tip varied from year to year. In 1999, the silk channel from husk tip to ear tip of Tex6 was significantly longer than that of B73 but the opposite was true in 2000. A significantly greater length of the ear tips of Tex6 ears did not fill compared with B73 ears in both years, but this did not appear to greatly influence the numbers of kernels damaged, as few *H. zea* penetrated the silk channel from the husk tip to the filled kernels of milk stage Tex6 in 1999, and the distance from the husk tip to the filled kernels of Tex6 (length of silk channel from husk

Table 2. Ear parameters and insect damage to milk stage ears in field studies of Tex6 and B73 maize inbreds at Peoria, IL, in 1999 (natural infestation) and 2000 (artificial infestation)

Insect and ear parameters	B73	Tex 6	*F*	χ^2	df	*P*
1999						
% incidence of caterpillar presence	86.4	85.0		0.03	1	0.86
% ears with kernel damage (if present)	84.0	15.0		40.0	1	<0.01
No. kernels damaged per ear (when damage present)	48.4 ± 4.2	13.4 ± 32.0	12.7		1, 41	<0.01
Weights of caterpillars, mg	324.8 ± 40.8	115.8 ± 31.2	11.1		1, 75	<0.01
Caterpillars per ear	1.3 ± 0.1	0.9 ± 0.1	8.54		1, 83	<0.01
Distance penetrated into silk channel from husk tip to ear tip, mm	60.0 ± 2.4	26.8 ± 2.9	79.0		1, 71	<0.01
Silk channel length, mm	60.0 ± 2.4	91.0 ± 3.9	48.3		1, 82	<0.01
Length of ear tip unfilled, mm	16.3 ± 1.2	36.3 ± 2.5	57.4		1, 84	<0.01
2000						
% incidence of caterpillar presence	100.0	100.0		0.00	1	1.00
% ears with kernel damage (if present)	87.5	64.3		2.25	1	0.13
No. kernels damaged per ear (when damage present)	26.4 ± 2.8	5.9 ± 0.7	33.6		1, 21	<0.01
Weights of caterpillars, mg	200.2 ± 32.4	68.2 ± 26.2	5.50		1, 43	0.02
Caterpillars per ear	2.2 ± 0.3	1.3 ± 0.2	6.51		1, 28	0.02
Distance penetrated into silk channel from husk tip to ear tip, mm	86.2 ± 4.4	46.4 ± 5.6	31.6		1, 28	<0.01
Silk channel length, mm	86.2 ± 4.4	53.2 ± 4.4	27.3		1, 28	<0.01
Length of ear tip unfilled, mm	29.7 ± 2.0	36.8 ± 1.7	7.00		1, 28	0.01

Chi-square values pertain to percentages analyzed by chi-square analysis, and *F* values pertain to means ± standard error values analyzed by ANOVA.

Table 3. Insect damage from natural infestations to harvest stage ears in field studies of Tex6 and B73 maize inbreds at Peoria, IL, in 1999 and 2000

Insect and ear parameters	B73	Tex6	*F*	χ^2	df	*P*
	1999					
% incidence of caterpillars	80.0	68.2		1.63	1	0.20
No. of kernels damaged per ear (if damage was present)	48.9 ± 3.9	13.9 ± 1.0	66.2		1, 30	<0.01
% incidence of symptomatic *Fusarium* associated with caterpillars	75.0	50.0		4.11	1	0.04
No. of kernels per ear with symptomatic *Fusarium* (if present)	14.9 ± 1.7	7.0 ± 1.0	12.2		1, 25	<0.01
% incidence symptomatic *Fusarium* not associated with caterpillars	10.0	15.0		0.13	1	0.71
	2000					
% incidence of caterpillars	24.8	20.8		0.40	1	0.53
No. of kernels damaged per ear (if damage was present)	21.7 ± 2.4	11.7 ± 1.5	11.3		1, 22	<0.01
% incidence of symptomatic *Fusarium* associated with caterpillars	24.8	11.1		4.17	1	0.04
No. of Kernels per ear with symptomatic *Fusarium* (if present)	8.2 ± 1.2	3.2 ± 1.2	8.54		1, 22	<0.01
% incidence symptomatic *Fusarium* not associated with caterpillars	14.5	5.6		3.24	1	0.07

Chi-square values pertain to percentages analyzed by chi-square analysis, and *F* values pertain to means ± standard error values analyzed by ANOVA.

tip to ear tip plus length of unfilled ear at tip) was equal to that for B73 in 2000.

At harvest, incidence of caterpillar (nearly all *H. zea* except for a few *O. nubilalis*) damage in the two types of inbred ears was similar for both years, and thus followed the trend noted for the milk stage ears (Table 3). The number of harvest stage kernels damaged per ear by caterpillars was significantly lower for Tex6 compared with B73 ears, as was found in milk stage samples. The incidence of ears with kernels symptomatically infected by *Fusarium* sp. fungi was significantly lower in Tex6 compared with B73 ears in both years. The numbers of kernels per ear symptomatically infected by *Fusarium* spp. fungi (when present) were significantly lower for Tex6 compared with B73 ears in both years. Sap beetle damaged kernels were the primary source of *Fusarium* damaged kernels not associated with caterpillar damage, and occurred at about the same rate for both inbreds in both years.

Laboratory Experiments. There were no significant differences in mortality of caterpillars or beetles caged with milk stage kernels of either inbred in either year (Table 4). There were no significant differences in weights of surviving caterpillars caged with kernels of the two inbreds in either year. However, Tex6 silks were often significantly more toxic than B73 silks to the caterpillars tested (Table 5). In 1999, Tex6 silks killed significantly more *H. zea* and *O. nubilalis* compared with B73 silks. Surviving *H. zea* and *S. frugiperda* fed Tex6 silks were significantly smaller than survivors fed B73 silks. In 1999, surviving caterpillars caged with Tex6 silks gained little or no weight compared with weights at hatch of ≈0.05 mg. In 2000, significantly more *H. zea* larvae fed Tex6 silks died, and survivors weighed significantly less compared with larvae fed B73 silks. However, there were no differences in mortality or survivor weight for *S. frugiperda* larvae fed Tex6 or B73 silks in 2000. Caterpillars (*H. zea*) fed darker colored pollinated Tex6 silks had significantly higher rates of mortality both in 1999 (53.1% versus 21.1%, respectively, $P = 0.01$, $\chi^2 = 6.35$, df = 1) and in 2000 (47.4% versus 15.8% mortality, $P = 0.04$, $\chi^2 = 4.38$,

Table 4. Effect of Tex6 and B73 milk stage kernels on caterpillars in 1999 and 2000 laboratory studies

Species	% mortality		χ^2	*P*	Weight, mg		*F*	df	*P*
	Tex6	B73			Tex6	B73			
				1999					
Corn earworm	5.0	0.0	0.97	0.32	2.1 ± 0.2	1.8 ± 0.2	0.82	1, 35	0.63
Fall armyworm	0.0	5.0	1.03	0.31	1.6 ± 0.2	1.8 ± 0.3	0.12	1, 36	0.73
European corn borer	15.8	5.3	1.12	0.29	0.6 ± 0.1	0.7 ± 0.1	0.23	1, 32	0.64
C. freemani	0.0	0.0	0.00	1.00	ND	ND			
Dusky sap beetle	10.0	0.0	2.10	0.15	ND	ND			
				2000					
Corn earworm	10.0	22.2	1.06	0.30	4.5 ± 1.1	4.5 ± 0.7	0.00	1, 29	0.98
Fall armyworm	5.0	0.0	1.03	0.31	2.7 ± 0.5	4.2 ± 0.6	3.66	1, 37	0.06
C. freemani	0.0	0.0	0.00	1.00	ND	ND			

ND, not determined. Degrees of freedom for chi-square analyses are all 1.

Table 5. Effect of Tex6 and B73 silks on caterpillars in 1999 and 2000 laboratory studies

	% mortality		χ^2	P	Weight, mg		F	df	P
	Tex6	B73			Tex6	B73			
				1999					
Corn earworm	52.6	6.7	9.17	<0.01	0.04 ± 0.04	0.71 ± 0.19	7.44	1, 21	0.01
Fall armyworm	31.5	10.5	2.63	0.10	0.09 ± 0.03	0.37 ± 0.10	5.31	1, 28	0.03
European corn borer	73.3	5.6	18.1	<0.01	0.06 ± 0.02	0.18 ± 0.04	2.86	1, 20	0.10
				2000					
Corn earworm	43.4	5.6	9.17	<0.01	0.27 ± 0.03	0.52 ± 0.08	4.42	1, 27	0.04
Fall armyworm	5.0	0.0	1.41	0.23	0.29 ± 0.03	0.21 ± 0.02	0.57	1, 32	0.57
European corn borer	ND	ND			ND	ND			

ND, not determined. Degrees of freedom for chi-square analyses are all 1.

df = 1) compared with those fed lighter colored unpollinated silks.

Discussion

Examples Where Insect Resistance Has Reduced Mycotoxins in Corn. Insect damage by caterpillars such as *H. zea* can facilitate the entry of mycotoxigenic fungi, and thereby greatly increase the levels of mycotoxins (review, Dowd 1998). Corn varieties with better husk coverage and longer/tighter silk channels (from husk tip to ear tip) can significantly reduce aflatoxin levels in the southeast by excluding damaging insects from the kernels (Barry et al. 1986; McMillian et al. 1985, 1987). However, ears with this type of husk coverage are more susceptible to *Fusarium* molds due to slow dry down in more northerly corn growing regions (Trenholm et al. 1989). Transgenic insect resistance due to expression of the Bt crystal protein, when expressed at high levels in silks and ears, as well as green tissue, can greatly reduce fumonisin levels compared with corresponding nontransgenic hybrids when *O. nubilalis* occurs at high levels (Munkvold et al. 1999) and is the predominant pest (Dowd 2000). However, reductions in fumonisin levels for Bt versus non-Bt hybrids have been much lower when *H. zea* is the major pest present (Dowd 2000, 2001). Reductions in *Aspergillus* ear rot or aflatoxin production in Bt relative to non-Bt inbreds or hybrids have generally been poorer than for fumonisin (e.g., Windham and Williams 1998). Thus, insect resistant corn germplasm can help to reduce levels of mycotoxins, depending on the range of insects it affects, and we would expect the silk resistance to caterpillars noted in the current study to also help reduce mycotoxin levels indirectly through insect control.

Tex6 Insect Resistance. The current study indicates Tex6, which has *A. flavus*-resistant ears (Campbell and White 1995, Hamblin and White 2000) and silks (McGee et al. 1995), and leaves resistant to southern corn leaf blight, *Cochliobolus heterostrophus* (Drechs.) Drechs. (D.G.W., unpublished data), compared with B73, also has silk and leaf resistance to several species of insects. The ear resistance to *Aspergillus* ear rot and aflatoxin production of Tex6 (Campbell and White 1995, Hamblin and White 2000) appears to be due to the presence of a novel chitinase (Moore et al. 1999). Chitinases are potentially useful in controlling insects (Kramer et al. 1997). However, milk stage kernels of Tex6 containing this chitinase do not appear to be directly toxic to the caterpillars tested, whether due to insufficient levels and/or insensitivity of the insects over the period tested.

The most insect resistant tissues of Tex6 in the current study were the silks. This conclusion is most strongly supported by field experiments in 1999, when *H. zea* caterpillars did not penetrate the silk channel of the Tex6 inbreds to the same degree as that of the B73 inbreds, and laboratory assays with Tex6 silks, which were more toxic to *H. zea* larvae than B73 silks in both years. Although some variation of the degree of silk resistance was noted in laboratory studies from year to year and species to species, this would not be unexpected based on the variety of environmental factors that can influence resistance (e.g., Tingey and Singh 1980). Year to year environmental variation may also explain reductions in activity to *S. frugiperda* from 1999 to 2000, which may be less sensitive or sensitive to different resistance factors present compared with *H. zea.*

Reduced milk stage kernel damage of Tex6 inbreds can also be explained by the silk resistance. In addition, to higher mortality which would reduce numbers per ear, development of survivors would have been slowed down so that smaller larvae encountered kernels and thus did less damage, a supposition that was also supported by the fewer numbers and smaller weights of larvae recovered from Tex6 compared with B73 ears. High levels of Bt crystal protein expressed in silks but not kernels due to a pith promoter were also able to prevent kernel feeding by *O. nubilalis* larvae such that no kernel damage occurred compared with the corresponding non-Bt inbred (Dowd 2000).

Based on experiments in the current study when unpollinated silks and pollinated silks of the same age were fed to caterpillars, pollination appeared to enhance the resistance of the Tex6 silks. Although this comparison was not performed with B73 silks in the current study, recent studies have indicated no significant differences in mortality or survivor weight of *H. zea* larvae fed silks from pollinated versus unpollinated kernels from milk stage B73 ears (P.F.D., unpublished data). Pollination state has also influenced silk resistance to caterpillars in other studies. For ex-

ample, 10-d-old pollinated silks of Zapalote Chico were much more susceptible to feeding by *H. zea* larvae than silks from unpollinated ears (Wiseman and Snook 1995).

Considering the toxicity of Tex6 silks to *H. zea* observed in our laboratory studies, it initially appears unclear why caterpillars were often found and damage occurred to Tex6 ears in our field studies. It is possible that oviposition by *H. zea* adults in 1999 occurred just at pollination, so larvae were able to feed on silks before levels of the resistance factors built up. It is also possible the larvae fed somewhat selectively on unpollinated silks, and thereby avoided the more toxic pollinated silks.

The degree of activity of the Tex6 silks against caterpillars we observed appears to be comparable to that of some the most highly resistant silks reported previously, although differences in the design of prior studies compared with the present one make direct comparisons difficult. Silks of Zapalote Chico (PI 217413) can greatly retard growth of *H. zea* larvae, but apparently do not cause significant mortality to neonates when incorporated into diets (e.g., Wiseman and Snook 1995), while on ears (Josephson et al. 1966), or fed fresh (Bennett et al. 1967). However, one report indicated mortality values of 24% and 80% for neonate *H. zea* larvae fed on regularly replenished non-exposed silks of Zapalote Chico after 15 and 25 d, respectively (Wiseman et al. 1976). Earlier resistance studies identified a silk "lethal factor" active against *H. zea* in some lines of corn (Walter 1957), but stable lines containing this factor were never developed or maintained (Walter 1957, Luckmann et al. 1964). Because these original lines were derived from southern and/or Illinois germplasm (Walter 1957), it is possible this factor has been reestablished in the Tex6 inbred, which is also derived from this type of germplasm (Hamblin and White 2000), although the obscurity of the original source materials make this difficult to say for certain.

In conclusion, the resistance to insects noted for the Tex6 inbred in the current study may be of considerable benefit in controlling mycotoxins in corn if it segregates with the fungal resistance during selective breeding. Resistance to *H.zea* is particularly valuable, as these insects can cause losses of over 15% in field corn in the southeastern United States and losses of 50% to sweet corn (Wiseman 1999). These insects have occurred more frequently in the major maize growing areas of the United States in the past few years (Dowd 2000), they or close relatives occur in many other maize growing regions in the world, and all of these caterpillars can greatly increase the levels of mycotoxins in corn (Dowd 1998). Evaluations of new germplasm, derived from Tex6 that was developed for *A. flavus* ear resistance, for resistance to insects are planned. The mechanisms of silk resistance are presently unknown, but appear to be primarily due to compounds other than maysin based on initial bioassays of solvent and chromatographically separated fractions of Tex6 silk extracts with and without maysin (including in the presence of oxidizing enzymes) (Dowd et al. 1999, 2000; M. A. Berhow et al., personal communication). It is possible new insect resistance mechanisms will be found in the Tex6 silks that will be amenable to genetic engineering strategies, whether proteins and/or secondary metabolites are involved.

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